ORIGINAL ARTICLE BACTERIOLOGY

Prevalence and characterization of extended-spectrum β-lactamase-producing clinical *Salmonella enterica* isolates in Dakar, Senegal, from 1999 to 2009

D. Harrois[1,2,*], S. Breurec[2,3,*], A. Seck[2], A. Delauné[1], S. Le Hello[1], M. Pardos de la Gándara[1], L. Sontag[1], J.-D. Perrier-Gros-Claude[2], J.-M. Sire[2], B. Garin[2,4] and F.-X. Weill[1]

1) *Institut Pasteur, Unité des Bactéries Pathogènes Entériques, Paris, France,* 2) *Institut Pasteur de Dakar, Unité de Bactériologie Médicale et Environnementale, Dakar, Senegal,* 3) *Institut Pasteur de Bangui, Laboratoire de Biologie Médicale, Bangui, République Centrafricaine and* 4) *Institut Pasteur de Madagascar, Laboratoire de Bactériologie Expérimentale, Antananarivo, Madagascar*

Abstract

A total of 1623 clinical isolates of *Salmonella* belonging to 229 serotypes were received by the Senegalese Reference Center for Enterobacteria from January 1999 to December 2009. The most common serotypes were Enteritidis (19% of the isolates), Typhi (8%), Typhimurium (7%) and Kentucky (4%). A significant increase in the prevalence of resistance to amoxicillin (0.9% in 1999 to 11.1% in 2009) and nalidixic acid (0.9% in 1999 to 26.7% in 2009) was observed in non-typhoidal *Salmonella* serotypes. For critically important antibiotics, notably ciprofloxacin and extended-spectrum cephalosporins (ESCs), the rates of resistance were low: 0.3% and 0.5%, respectively. Seven ESC-resistant *Salmonella* strains and three additional ESC-resistant strains from Senegal (1990) and Mali (2007) were studied to identify the genetic basis of their antibiotic resistance. All ESC-resistant strains produced an extended-spectrum β-lactamase (ESBL). These were CTX-M-15 (n = 6; 2000–2008), SHV-12 (n = 3; 2000–2001) and SHV-2 (n = 1; 1990). A large IncHI2 ST1 pK29-like plasmid was found in six strains (three producing SHV-12 and three CTX-M-15), whereas IncN and IncF plasmids were found in three strains and one strain, respectively. The association of plasmid-mediated quinolone resistance (PMQR) genes *qnrB1* and *aac(6′)-Ib-cr* was found in four ESBL-producing strains, leading to decreased susceptibility and even full resistance to ciprofloxacin (MIC range 0.75–2 mg/L) despite the absence of mutations in the quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB*, *parC* and *parE*. This association of ESBL and multiple PMQR mechanisms within the same strains is therefore a serious concern as it hampers the use of both ESCs and fluoroquinolones for severe *Salmonella* infections.

Keywords: AAC(6′)-Ib-cr, antimicrobial resistance, CTX-M, extended-spectrum β-lactamase, IncHI2 plasmid, IncN plasmid, Qnr, *Salmonella enterica*, Senegal, SHV
Original Submission: 5 February 2013; **Revised Submission:** 3 July 2013; **Accepted:** 14 July 2013
Editor: R. Cantón
Article published online: 18 July 2013
Clin Microbiol Infect 2014; **20:** O109–O116
10.1111/1469-0691.12339

Corresponding author: F.-X. Weill, Unité des Bactéries Pathogènes Entériques, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France
E-mail: francois-xavier.weill@pasteur.fr
*These authors contributed equally.

Introduction

Human *Salmonella* infections are generally either typhoid fever, a systemic disease caused by *S. enterica* serotypes Typhi, Paratyphi A, Paratyphi B (non-*d*-tartrate-fermenting variant) and Paratyphi C, or gastroenteritis caused by a large number of non-typhoidal *Salmonella* (NTS) serotypes. Typhoidal serotypes are human-restricted whereas NTS have large animal reservoirs. Although most salmonellosis due to NTS is self-limiting, serious complications, including systemic infection and death, can occur. Such infections have consistently been reported as a leading cause of bacteraemia in Africa and are associated with a high risk of death [1]. As rates of resistance to all classes of antibiotics have increased throughout the world, conventional antibiotics such as ampicillin, chloramphenicol and cotrimoxazole are no longer

the appropriate choices and extended-spectrum cephalosporins (ESCs) and fluoroquinolones have become standard for first-line empirical treatment in children and adults, respectively [2]. Recently, ESC-resistant (ESC^R) *Salmonella* populations have emerged and spread over all continents, including Africa [2–15]. This resistance is mainly mediated by acquired extended-spectrum β-lactamase (ESBL) genes carried by mobile genetic elements such as plasmids and transposons. This situation is of great concern, as ESBL enzymes can hydrolyze almost all β-lactams (except carbapenems and cephamycins), and are frequently associated with genes conferring resistance to several other classes of antibiotics. Recently, plasmid-mediated quinolone resistance (PMQR) has emerged in Enterobacteriaceae. Four PMQR mechanisms have been described: Qnr, AAC(6′)-Ib-cr (AACA4-cr), OqxAB and QepA, which mediate target protection (Qnr), drug modification (AAC(6′)-Ib-cr) and drug efflux (OqxAB and QepA) [16]. These mechanisms result in an increase of the minimum inhibitory concentration (MIC) of fluoroquinolones, thereby facilitating the selection of mutants with higher levels of resistance in the presence of quinolones through sequential chromosomal mutations in genes coding for the target enzymes, DNA gyrase and/or DNA topoisomerase IV [16].

Few data are available regarding the prevalence of ESC^R *Salmonella* strains in Africa or their antibiotic resistance gene content and their genetic support. Such information is important for an understanding of the spread of multidrug-resistant *Salmonella* spp.. Here, we report the prevalence of resistance to antibiotics in *Salmonella* spp. isolated from Senegalese patients between 1999 and 2009, and the genetic basis for this antibiotic resistance. Three additional ESC^R strains from Senegal (1990) and from Mali (2007) were also included to provide a better description of circulating ESBL-producing *Salmonella* strains in West Africa.

Materials and Methods

Bacterial strains, serotyping and susceptibility

A total of 1623 *S. enterica* clinical isolates were received between January 1999 and December 2009 by the Senegalese Reference Center for Enterobacteria (Institut Pasteur, Dakar, Senegal) from four major public and private clinical laboratories (Hôpital Aristide Le Dantec, Hôpital Principal, Institut Pasteur and Bio24) located in Dakar. If more than one isolate with the same serotype and antimicrobial resistance phenotype was recovered from the same patient, only the first was included. Epidemiological data (date and site of isolation, age and gender of the patient) were recorded for each ESC^R isolate. ESC^R *Salmonella* strains from other collections were also included in this study: one strain (09-7364) isolated in Senegal in 1990 (Poitiers University Hospital collection, France) and two strains (07-0319, 07-1331) acquired in Mali in 2007 (the collection of the French National Reference Center for *Salmonella*, Institut Pasteur, Paris).

Strains were serotyped on the basis of somatic O and both phase 1 and phase 2 flagellar antigens by agglutination tests with antisera (Bio-Rad, Marnes-La-Coquette, France) as specified by the White-Kauffmann-Le Minor scheme [17]. Antibiotic susceptibility to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, cefalotin, cefoxitin, cefotaxime, ceftazidime, amikacin, tobramycin, gentamicin, nalidixic acid, ciprofloxacin, chloramphenicol, sulphonamides, cotrimoxazole and tetracycline was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad) according to the guidelines of the French Society for Microbiology. (http://www.sfm-microbiologie.org/UserFiles/file/CASFM/casfm_2011.pdf).

All strains of *Salmonella* showing resistance to cefotaxime and/or ceftazidime were selected for further analysis. Additional testing was carried out on these strains: (i) susceptibility to piperacillin, piperacillin/tazobactam, imipenem and streptomycin was determined by the disk diffusion method as described above; (ii) they were tested for an ESBL enzyme by the double disk synergy method [18]; and (iii) MICs for nalidixic acid, ciprofloxacin, ceftriaxone and ceftazidime were determined by using Etest strips (bioMérieux, Marcy L'Etoile, France). The CA-SFM cut-off values were used for categorization. Susceptible strains were defined by MIC $\leq$ 8 mg/L for nalidixic acid, MIC $\leq$ 0.5 mg/L for ciprofloxacin, MIC $\leq$ 1 mg/L for ceftriaxone and MIC $\leq$ 4 mg/L for ceftazidime, and resistant strains by MIC $>$ 16 mg/L for nalidixic acid, MIC $>$ 1 mg/L for ciprofloxacin, MIC $>$ 2 mg/L for ceftriaxone and MIC $>$ 4 mg/L for ceftazidime.

Characterization of resistance determinants in ESC^R strains

Total DNA was extracted using the Instagene™ Matrix kit (Bio-Rad) according to the manufacturer's recommendations. The resistance genes, bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, $bla_{OXA\text{-}1}$ group, *qnrA*, *qnrB*, *qnrS*, *qnrD*, *aac(6′)-Ib* and *qepA*, were amplified by PCR from DNA from all ESC^R strains as described previously [5,19]. For all *qnr*-positive ESC^R strains, the quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB*, *parC* and *parE* (encoding subunits of the DNA gyrase and topoisomerase IV) was sequenced, as described previously [19]. The nucleotide and deduced amino acid sequences were analysed and compared with sequences available through the Internet on the National Center for Bio-technology Information web site (http://www.ncbi.nlm.nih.gov).

Molecular typing

Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested chromosomal DNA was performed for ESC^R strains of serotypes Kentucky and Agona, as previously described [6].

Resistance transfer determination of ESC^R strains

Resistance transfer experiments were carried out on solid media using either *Escherichia coli* K12 J5 resistant to sodium azide or *E. coli* C1a resistant to nalidixic acid as the recipient strain [5,10]. Transconjugants were selected on Drigalski agar (Bio-Rad) supplemented with sodium azide (500 mg/L) and ceftriaxone (2 mg/L) or ceftazidime (4 mg/L) or ciprofloxacin (0.06 mg/L). Three *E. coli* transconjugants were arbitrarily selected in each experiment.

Plasmid analysis

Plasmid DNA extracted by alkaline lysis was analysed by electrophoresis in 0.8% agarose gels [5]. We used S1 nuclease treatment and PFGE to determine the sizes of bacterial plasmids, in parental and transconjugant strains, as described previously [10]. PCR-based replicon-typing analysis was performed as described previously [20]. Subtyping of plasmids was carried out by plasmid MLST (pMLST) (http://pubmlst.org/plasmid/) and multiplex PCR [21].

Statistical analysis

The chi-square test for trend was used to compare rates of resistance to antibiotics during the study period. p values of ≤ 0.05 were considered statistically significant. All analyses were performed using STATA 12.0 (Stata Corporation, College Station, TX, USA).

Results

Serotype distribution and antimicrobial susceptibility data

A total of 1623 independent isolates of *Salmonella* belonging to 229 serotypes were collected during the 11-year study period. The most common serotypes were Enteritidis (19% of the isolates), Typhi (8%), Typhimurium (7%) and Kentucky (4%) (Table 1). Most of the isolates were from stools (n = 1281, 78.6%) and blood (n = 191, 11.7%). Among *Salmonella* blood isolates, 118 (61.8%) were NTS, with Enteritidis (30.5% of the isolates) and Typhimurium (15.8%) being the predominant serotypes. The high prevalence of serotypes Kentucky in 2003 (12% of the total) and Poona (17%) in 2006 were due to documented food poisoning episodes.

Overall, the 1476 NTS isolates had low or moderate resistance to antibiotics. However, a significant increase in the prevalence of resistance to amoxicillin (0.9% in 1999 to 11.1%

TABLE 1. Distribution of the ten most frequent *Salmonella enterica* serotypes in Dakar, Senegal, 1999–2009

1999 n = 139	%	2000 n = 131	%	2001 n = 215	%	2002 n = 320	%	2003 n = 146	%	2004 n = 205	%	2005 n = 142	%	2006 n = 116	%	2007 n = 101	%	2008 n = 55	%	2009 n = 53	%	1999–2009 n = 1623	%
Enteritidis	18	Enteritidis	30	Enteritidis	22	Enteritidis	22	Kentucky	12	Enteritidis	31	Enteritidis	17	Poona	17	Schwarzengrund	17	Typhimurium	15	Typhimurium	15	Enteritidis	19
Typhi	17	Typhi	10	Typhi	8	Typhi	12	Enteritidis	12	Virchow	5	Brancaster	5	Virchow	5	Typhimurium	5	Typhi	9	Enteritidis	15	Typhi	8
Othmarschen	4	Typhimurium	10	Kentucky	6	Typhimurium	11	Typhi	10	Give	4	Bredeney	5	Kentucky	4	Typhi	5	Chester	5	Paratyphi A	8	Typhimurium	7
Typhimurium	4	Kentucky	5	Hadar	5	Virchow	4	Typhimurium	6	Corvallis	3	Typhi	4	Montevideo	4	Enteritidis	5	Enteritidis	5	Kentucky	6	Kentucky	4
Paratyphi A	3	Agona	4	Typhimurium	4	Altona	3	Bredeney	5	Montevideo	3	Kentucky	4	Nima	4	Oranienburg	5	Grumpensis	5	Johannesburg	6	Virchow	3
Montevideo	3	Keurmassar	4	Chester	3	Hadar	2	Give	3	Typhimurium	3	Senftenberg	4	Oranienburg	4	Kentucky	4	Virchow	5	Give	6	Poona	3
Kentucky	3	Othmarschen	2	Agona	2	Kentucky	2	Poona	3	Oranienburg	2	Tilene	4	Enteritidis	3	Poona	4	Schwarzengrund	4	Oranienburg	4	Hadar	2
Tsevie	2	Virchow	2	Newlands	2	Montevideo	2	Reading	3	Typhi	2	Corvallis	3	Schwarzengrund	3	Paratyphi A	3	Altona	4	Brancaster	4	Give	2
Livingstone	2	Albany	2	Montevideo	2	Give	2	Schwarzengrund	3	Johannesburg	2	Hadar	3	Brandenburg	3	Johannesburg	3	Paratyphi A	3	Muenster	4	Oranienburg	2
Lika	2	Newlands	2	Virchow	2	Bredeney	2	Hadar	2	Poona	2	Muenster	2	Hadar	3	Molade	3	Give	3	Carmel	4	Bredeney	2

TABLE 2. Percentage of resistance to specific antibiotics in non-typhoidal *Salmonella* serotypes in Dakar, Senegal, from 1999 to 2009

Year	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	
Number of isolates tested	**112**	**119**	**198**	**281**	**132**	**202**	**134**	**114**	**93**	**46**	**45**	**p value**
Amoxicillin	0.9	2.5	1.0	1.1	0.8	7.9	2.2	1.8	4.3	8.7	11.1	<0.0001
Cefoxitin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	>0.5
Cefotaxime	0.0	2.5	0.5	0.4[b]	0.0	0.0	0.0	0.0	0.0	4.3	2.2	>0.5
Ceftazidime	0.0	2.5	0.5	0.4[b]	0.0	0.0	0.0	0.0	0.0	4.3	2.2	>0.5
Amikacin	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	>0.5
Tobramycin	0.0	7.6	1.0	1.4	0.0	0.5	0.0	0.0	0.0	4.3	8.7	>0.5
Gentamicin	0.0	7.6	1.0	1.4	0.0	0.5	0.0	0.0	0.0	8.7	4.3	>0.5
Nalidixic acid	0.9	0.0	2.5	1.4	0.8	4.0	8.2	4.4	4.3	15.2	26.7	<0.0001
Ciprofloxacin	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	4.4	>0.5
Chloramphenicol	[a]	12.6	3.5	6.0	1.5	2.0	0.7	2.6	3.2	6.5	4.4	>0.5
Sulphonamide	[a]	[a]	[a]	[a]	[a]	14.9	21.6	18.4	29.0	37.0	33.3	>0.5
Cotrimoxazole	5.4	21.0	9.6	10.0	6.1	7.4	9.7	7.0	6.5	17.4	8.9	>0.5
Tetracycline	67.0	26.1	15.7	8.9	5.3	5.9	14.2	6.1	10.8	10.9	28.9	>0.5

[a]Not tested.
[b]This resistance corresponds to the CTX-M-15-producing *S. enterica* serotype Kentucky strain M08028, which was erroneously reported in reference 5 as having been isolated in 2001.

in 2009) ($p < 0.0001$) and nalidixic acid (0.9% in 1999 to 26.7% in 2009) ($p < 0.0001$) was observed during the study period (Table 2). Four (0.3% of the total) NTS isolates belonging to serotypes Keurmassar (2000, $n = 1$) (MIC of 1.5 mg/L) [4], Enteritidis (2007, $n = 1$) (MIC of 1.25 mg/L) and Kentucky (2009, $n = 2$) (MIC of 3 and >12 mg/L) were resistant to ciprofloxacin; one of these isolates was ESC^R (Keurmassar). All the serotype Typhi ($n = 127$), Paratyphi A ($n = 19$) and Paratyphi B ($n = 1$) isolates were susceptible to all antibiotics tested, except for five serotype Paratyphi A isolates: three were resistant to tetracycline, one was resistant to amoxicillin and another one to cotrimoxazole. Eight NTS strains (0.5% of the total) belonging to serotypes Keurmassar ($n = 1$, 2000) [4], Kentucky ($n = 2$, 2000 and 2002) [5], Agona ($n = 2$, 2000 and 2001), Grumpensis ($n = 1$, 2008), Typhimurium ($n = 1$, 2008) and Carmel ($n = 1$, 2009) were resistant to all the β-lactams tested, except for cefoxitin, piperacillin-tazobactam and imipenem. Both serotype Kentucky strains were previously shown to display identical PFGE profiles and antimicrobial susceptibility profiles [5], whereas the two serotype Agona strains had two similar but not identical PFGE profiles (not shown) and different antimicrobial susceptibility profiles. Therefore, only one serotype Kentucky strain but both serotype Agona strains were selected for further molecular investigation. These seven ESC^R strains, as well as three additional ESC^R strains from Mali and Senegal (belonging to serotypes Miami, Havana and Telelkebir), scored positive in double-disk synergy tests and showed resistance to several classes of antibiotics (Table 3). In particular, five strains were resistant to nalidixic acid and three of these five were resistant to ciprofloxacin (MIC 1.5–2 mg/L); three were resistant to all but one of the aminoglycosides (amikacin) tested.

Resistance determinants in ESC^R strains

Among the 10 ESC^R isolates, the $bla_{CTX\text{-}M\text{-}15}$ gene was detected in all of those isolated between 2007 and 2009 ($n = 5$), and also in one strain isolated in 2000, whereas $bla_{SHV\text{-}12}$ was detected in three strains isolated in 2000 and 2001, and $bla_{SHV\text{-}2}$ in the oldest strain from 1990 (Table 3).

The *qnr* gene was present in five ESC^R isolates (62%): two were *qnrB2* and three were *qnrB1*. The *qnrB1* gene was associated with the $bla_{CTX\text{-}M\text{-}15}$ gene, and *qnrB2* with the $bla_{SHV\text{-}12}$ gene. No *qnr* was found in the oldest strain that produces SHV-2. The five *qnr*-positive strains had wild-type alleles in the QRDR of *gyrA*, *gyrB*, *parC* and *parE*. They displayed MICs to nalidixic acid in the range 24–64 mg/L, and MICs to ciprofloxacin of 0.25–2 mg/L. According to CA-SFM breakpoints, three were classified as resistant to ciprofloxacin (MICs 1.5–2 mg/L), one as susceptible (MIC of 0.25 mg/L) and one as intermediate (MIC of 0.75 mg/L) (Table 3). The presence of both *qnr* and *aac(6')-Ib-cr* was associated with higher MICs to ciprofloxacin (MICs 0.75–2 mg/L).

Genetic support of resistance determinants

The conjugation experiments showed that all the ESBL genes and *qnr* genes were transferred into *E. coli* recipients (Table 3).

The $bla_{CTX\text{-}M\text{-}15}$ genes were located on plasmids of the incompatibility groups HI2 ($n = 3$; all assigned to ST1), *n* ($n = 2$; both assigned to ST6) and F ($n = 1$; assigned to F2:A-:B-). Both *qnrB1* and the *aac(6)'-Ib-cr* genes were on large IncHI2 plasmids (280–340 kb) also carrying the $bla_{CTX\text{-}M\text{-}15}$ gene. The penicillinase $bla_{OXA\text{-}1}$ and bla_{TEM} genes were also present on the large IncHI2 plasmid.

For the three SHV-12-producing *S. enterica* serotype Agona and Keurmassar strains and their transconjugants, both one IncHI2 ST1 plasmid and a second plasmid (IncI1 for serotype

TABLE 3. **Molecular characteristics of the ten extended-spectrum cephalosporin-resistant *Salmonella* strains studied and their *Escherichia coli* transconjugants**

Strain	Year Country	Source	MIC[a] (mg/L)				Additional resistances	ESBL[b]/PMQR[c]	Inc group[g]	Size of plasmid (kb)
			CAZ	CRO	NA	CIP				
Salmonella ser. Miami 09-7364	1990 Senegal	Unknown	24	64	4	0.02	CHL	SHV-2/none	N	50
E. coli TC1 p09-7364			4	1.5	ND	ND	CHL	SHV-2/none	N (ST16)	50
Salmonella ser. Keurmassar CIS 9118/00	2000 Senegal	Human blood[e]	>256	8	24	1.5	S K TM GM SSS CHL TE	SHV-12/QnrB2, Aac(6')-Ib-cr	H12, F	120, 300
E. coli TC p9118/00-1			16	2	24	0.25	K TM GM SSS CHL TE	SHV-12/QnrB2	H12 (ST1), F (F31:A4:B1)	120, >300
Salmonella ser. Kentucky K18109	2000 Senegal	Human stool[f]	>256	>256	1.5	0.05	S K TM GM SSS TMP TE	CTX-M-15	N	30, 70
E. coli TC pK18109-1[d]			>256	>256	ND	ND	S K TM GM SSS TMP TE	CTX-M-15	N (ST6)	70
Salmonella ser. Agona K1002128	2000 Senegal	Human stool[e]	256	16	4	0.08	S GM SSS TMP CHL	SHV-12	H12, I1	90, 300
E. coli TC pK1002128-1[d]			256	16	ND	ND	S GM SSS TMP CHL	SHV-12	H12 (ST1), I1 (ST26)	90, 300
Salmonella ser. Agona L0411198	2001 Senegal	Human blood[e]	>256	16	24	0.25	S K TM GM SSS TMP CHL TE	SHV-12/QnrB2	H12, I1	90, 300
E. coli TC pL0411198-1			>256	16	12	0.12	S K TM GM SSS TMP CHL TE	SHV-12/ QnrB2	H12 (ST1), I1 (ST26)	90, >300
Salmonella ser. Havana 07-0319	2007 Mali	Human stool[f]	>256	>256	64	2	S K TM GM SSS TMP CHL TE	CTX-M-15/QnrB1, Aac(6')-Ib-cr	H12	340
E. coli TC p07-0319-1			16	64	16	1	S K TM SSS TMP CHL TE	CTX-M-15/QnrB1, Aac(6')-Ib-cr	H12 (ST1)	340
Salmonella ser. Telelkebir 07-1331	2007 Mali	Human blood[f]	48	>256	48	1.5	S K TM GM SSS TMP CHL TE	CTX-M-15/QnrB1, Aac(6')-Ib-cr	H12	350
E. coli TC p07-1331-3			32	96	24	1.5	S K TM GM SSS TMP CHL TE	CTX-M-15/QnrB1, Aac(6')-Ib-cr	H12 (ST1)	350
Salmonella ser. Grumpensis 08-3663	2008 Senegal	Human CSF[f]	48	>256	32	0.75	S TM GM SSS TMP CHL TE	CTX-M-15/QnrB1, Aac(6')-Ib-cr	H12	280
E. coli TC p08-3663-1			12	64	8	0.5	S TM GM SSS TMP CHL TE	CTX-M-15/QnrB1, Aac(6')-Ib-cr	H12 (ST1)	280
Salmonella ser. Typhimurium 08-3664	2008 Senegal	Human blood[e]	48	>256	4	0.05	S K TM GM SSS TMP	CTX-M-15/none	N, FII_S, FIB_S	60, 100, 150
E. coli TC p08-3664-1[d]			24	>256	ND	ND	S K TM GM SSS TMP	CTX-M-15/none	N (ST6)	60
Salmonella ser. Carmel T0706277	2009 Senegal	Human stool	>256	>256	4	0.06	K TM GM	CTX-M-15/none	F	90
E. coli TC pT0706277-1			48	48	ND	ND	K TM	CTX-M-15/none	F (F2:A-:B-)	90

CAZ, ceftazidime; CRO, ceftriaxone; S, streptomycin; K, kanamycin; TM, tobramycin; GM, gentamicin; SSS, sulphonamides; TMP, trimethoprim; CHL, chloramphenicol; TE, tetracycline; NA, nalidixic acid; CIP, ciprofloxacin.
[a]Minimal inhibitory concentration; ND, not determined.
[b]Extended-spectrum β-lactamase.
[c]Plasmid-mediated quinolone resistance.
[d]*E. coli* C1a Nal^R, transconjugants indicated by TC prefix in the strain designation.
[e]Hospital-acquired.
[f]Not hospital-acquired.
[g]All IncHI2 ST1 plasmids had the same profile in the multiplex PCR assay (*hipA* positive, *smr0092* positive, *smr0183* positive).

Agona strains and IncF for serotype Keurmassar strain) were detected. In the absence of southern blot and hybridization with specific probes, we cannot determine the exact location of the bla_{SHV-12} gene; however, it is probable that this gene is carried by at least the IncHI2 plasmid, which was common to the three SHV-12-producing strains.

The *S. enterica* serotype Miami strain had a bla_{SHV-2} gene on an IncN (ST16) plasmid.

Discussion

In total, 1623 non-duplicate *Salmonella* spp. isolates were analysed during the study period. Overall, the resistance rates to all antibiotic classes were low or moderate although there was a worrying increase in the prevalence of resistance to certain first-line antibiotics during the study period. The prevalence of ESC^R isolates in our study was 0.5%, consistent with previous studies elsewhere in the world (0–2.4%) [2,22–24]. National surveillance systems are primarily based on a network of clinical laboratories that refer *Salmonella* isolates to public health laboratories for identification and susceptibility testing. However, such systems are lacking in most countries with inadequate healthcare systems. Consequently, many studies in Africa are not appropriately representative of the epidemiological situation nationally. Despite these limitations, the number of new cases of ESC^R *Salmonella* strains (novel associations of serotype, enzyme and country) seems to have increased across Africa since the first description in 1988 in Tunisia [3]. So far, 23 different *Salmonella* serotypes producing ESBLs have been described in studies of human isolates in West Africa [2,4–6], Maghreb [2,3,9,12,13,15], East Africa [2,8,10,11], South Africa [7,14] and Central Africa (S. Breurec, unpublished data). Typhimurium is the most frequently isolated ESBL-producing serotype in these ten African countries. ESBL-producing *Salmonella* can be acquired in the community [2,5,7,12] or in hospitals [2,8,9,13], in particular in paediatric wards and neonatology units.

Several reports also mention internationally adopted children from Mali or Ethiopia as carriers of ESBL-producing *Salmonella* of various serotypes [6,10,11]. Such strains may have been acquired in the orphanages where the children had stayed before adoption: the prevailing conditions, such as overcrowding and high ESCs pressure, may have favoured such strains. However, a study performed outside orphanages found that one of the ESBL-producing *Salmonella* strains (serotype Concord) circulating in the orphanage was also circulating in the general population of Ethiopia [25]. In our study, the bla_{SHV-2} gene was detected in the oldest strain from 1990, the bla_{SHV-12} gene in three strains isolated in the early 2000s, and the $bla_{CTX-M-15}$ gene in all the strains isolated since the mid 2000s. This shift from TEM/SHV to CTX-M, and in particular to CTX-M-15, is in accordance with other epidemiological studies of ESBL-producing *Salmonella* spp. in Africa [10].

The ESBL $bla_{CTX-M-15}$ gene was carried on plasmids of three different incompatibility groups, suggesting that the dissemination of $bla_{CTX-M-15}$ among *Salmonella* spp. in West Africa is not due to a single type of plasmid. These plasmids were different from the CTX-M-15/IncFII plasmid present in the ST131 *E. coli* clone disseminated worldwide [26]. The most frequent plasmid we found was a large IncHI2 ST1 similar to pK29 [21]. It was present in five different *Salmonella* serotypes from 2000 to 2008, two that produced SHV-12 and three that produced CTX-M-15. There is currently no IncHI2 ST1 plasmid carrying the $bla_{CTX-M-15}$ gene in the PubMLST database, and there are only two IncHI2 pK29-like plasmids carrying bla_{SHV-12} genes. These two plasmids were isolated from *E. coli* and *Enterobacter cloacae* from humans in France and Australia. The most probable explanation is that the pDLST/multiplex PCR subtyping scheme for IncHI2 is too recent (2010) to allow the database to be representative of circulating plasmids. Large IncHI2 plasmids carrying the $bla_{CTX-M-15}$ gene have already been identified in *S. enterica* serotype Concord from Ethiopia [10,27], in *E. coli*, *Klebsiella pneumoniae*, *K. oxytoca* and *E. cloacae* in Madagascar [28] and in *Enterobacter* spp. in Norway [29], and large IncHI2 plasmids carrying the bla_{SHV-12} have been found in *S. enterica* serotype Senftenberg from Egypt [27] and in *Enterobacter* spp. in Norway [29]. All these plasmids were isolated from human sources and were frequently associated with *qnrB1* and *aac(6′)-Ib-cr* genes. Frequent association of $bla_{CTX-M-15}$, *qnrB* and *aac(6′)-Ib-cr* genes has also been described in *K. pneumoniae* isolates from Maghreb, West Africa, Central Africa and East Africa, consistent with these resistance determinant genes being carried together on the same plasmid [30].

Such accumulation of PMQR determinants (i.e. both *qnrB1* and *aac(6′)-Ib-cr*) in ESBL-producing strains is a matter of concern as these determinants lead to decreased susceptibility and even full resistance to ciprofloxacin (MIC range 0.75–2 mg/L) despite the absence of mutations in the QRDR. However, the involvement of an efflux system such as AcrAB-TolC and OqxAB or the presence of the *qnrC* gene were not investigated and therefore cannot be excluded.

In conclusion, the data reported here add to the knowledge of the circulation of multidrug-resistant *Salmonella* populations in West Africa. Although rare, resistance to ESC and ciprofloxacin is present. Therefore, it seems important to continue monitoring antimicrobial susceptibility in *Salmonella* isolates from humans, foodstuffs and food animals in Senegal.

Acknowledgements

We thank the teams of the National Reference Center for *Salmonella*, Institut Pasteur in Paris (France), and of the Senegalese Reference Center for *Enterobacteria,* Institut Pasteur in Dakar (Senegal), for their technical help. We would also like to thank Christophe Burucoa (Poitiers University Hospital, France), who provided one strain included in this study.

Transparency Declaration

Dorothée Harrois was supported by a grant from 'La Fondation pour la Recherche Médicale'. The authors have no conflict of interests.

References

1. Tabu C, Breiman RF, Ochieng B *et al.* Differing burden and epidemiology of non-typhi *Salmonella* bacteremia in rural and urban Kenya, 2006–2009. *PLoS ONE* 2012; 7: e31237.
2. Arlet G, Barrett TJ, Butaye P, Cloeckaert A, Mulvey MR, White DG. *Salmonella* resistant to extended-spectrum cephalosporins: prevalence and epidemiology. *Microbes Infect* 2006; 8: 1945–1954.
3. Hammami A, Arlet G, Ben Redjeb S *et al.* Nosocomial outbreak of acute gastroenteritis in a neonatal intensive care unit in Tunisia caused by multiply drug resistant *Salmonell*a Wien producing SHV-2 beta-lactamase. *Eur J Clin Microbiol Infect Dis* 1991; 10: 641–646.
4. Cardinale E, Colbachini P, Perrier-Gros-Claude JD, Gassama A, Aïdara-Kane A. Dual emergence in food and humans of a novel multiresistant serotype of *Salmonella* in Senegal: *Salmonella enterica* subsp. *enterica* serotype 35:c:1,2. *J Clin Microbiol* 2001; 39: 2373–2374.
5. Weill FX, Perrier-Gros-Claude JD, Demartin M, Coignard S, Grimont PA. Characterization of extended-spectrum-beta-lactamase (CTX-M-15)-producing strains of *Salmonella enterica* isolated in France and Senegal. *FEMS Microbiol Lett* 2004; 238: 353–358.
6. Weill FX, Demartin M, Tandé D, Espié E, Rakotoarivony I, Grimont PA. SHV-12-like extended-spectrum beta-lactamase-producing strains of *Salmonella enterica* serotypes Babelsberg and Enteritidis isolated in France among infants adopted from Mali. *J Clin Microbiol* 2004; 42: 2432–2437.
7. Kruger T, Szabo D, Keddy KH *et al.* Infections with nontyphoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. *Antimicrob Agents Chemother* 2004; 48: 4263–4270.
8. Blomberg B, Jureen R, Manji KP *et al.* High rate of fatal cases of pediatric septicemia caused by Gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. *J Clin Microbiol* 2005; 43: 745–749.
9. Bouallègue-Godet O, Ben Salem Y, Fabre L *et al.* Nosocomial outbreak caused by *Salmonella enterica* serotype Livingstone producing CTX-M-27 extended-spectrum beta-lactamase in a neonatal unit in Sousse, Tunisia. *J Clin Microbiol* 2005; 43: 1037–1044.
10. Fabre L, Delauné A, Espié E *et al.* Chromosomal integration of the extended-spectrum beta-lactamase gene $bla_{CTX\text{-}M\text{-}15}$ in *Salmonella enterica* serotype Concord isolates from internationally adopted children. *Antimicrob Agents Chemother* 2009; 53: 1808–1816.
11. Hendriksen RS, Mikoleit M, Kornschober C *et al.* Emergence of multidrug-resistant *Salmonella* Concord infections in Europe and the United States in children adopted from Ethiopia, 2003–2007. *Pediatr Infect Dis J* 2009; 28: 814–818.
12. Bouchrif B, Le Hello S, Pardos M *et al.* Ceftazidime-resistant *Salmonella enterica*, Morocco. *Emerg Infect Dis* 2009; 15: 1693–1695.
13. Naas T, Bentchouala C, Cuzon G *et al.* Outbreak of *Salmonella enterica* serotype Infantis producing ArmA 16s RNA methylase and CTX-M-15 extended-spectrum beta-lactamase in a neonatology ward in Constantine, Algeria. *Int J Antimicrob Agents* 2011; 38: 135–139.
14. Hendriksen RS, Joensen KG, Lukwesa-Musyani C *et al.* Extreme drug resistant *Salmonella enterica* Senftenberg infections from patients in Zambia. *J Clin Microbiol* 2013; 51: 284–286.
15. Le Hello S, Harrois D, Bouchrif B *et al.* Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. *Lancet Infect Dis* 2013; 13: 672–679.
16. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin Microbiol Rev* 2009; 22: 664–689.
17. Grimont P, Weill FX, eds. *Antigenic formulae of the Salmonella serovars*, 9th edn. Paris, France: WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, 2007.
18. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10: 867–878.
19. Le Hello S, Hendriksen RS, Doublet B *et al.* International spread of an epidemic population of *Salmonella enterica* serotype Kentucky ST198 resistant to ciprofloxacin. *J Infect Dis* 2011; 204: 675–684.
20. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; 63: 219–228.
21. García-Fernández A, Carattoli A. Plasmid double locus sequence typing for IncHI2 plasmids, a subtyping scheme for the characterization of IncHI2 plasmids carrying extended-spectrum beta-lactamase and quinolone resistance genes. *J Antimicrob Chemother* 2010; 65: 1155–1161.
22. Pardos de la Gandara M, Seral C, Castillo Garcia J, Rubio Calvo C, Weill FX. Prevalence and characterization of extended-spectrum beta-lactamases-producing *Salmonella enterica* isolates in Saragossa, Spain (2001–2008). *Microb Drug Resist* 2011; 17: 207–213.
23. Monno R, Rizzo C, De Vito D *et al.* Prevalence, antimicrobial resistance, and extended-spectrum beta-lactamases characterization of *Salmonella* isolates in Apulia, southern Italy (2001–2005). *Microb Drug Resist* 2007; 13: 124–129.
24. Egorova S, Kaftyreva L, Grimont PA, Weill FX. Prevalence and characterization of extended-spectrum cephalosporin-resistant non-typhoidal *Salmonella* isolates in adults in Saint Petersburg, Russia (2002–2005). *Microb Drug Resist* 2007; 13: 102–107.
25. Beyene G, Nair S, Asrat D, Mengistu Y, Engers H, Wain J. Multidrug resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia. *J Infect Dev Ctries* 2011; 5: 23–33.
26. Coque TM, Novais A, Carattoli A *et al.* Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg Infect Dis* 2008; 14: 195–200.
27. Veldman K, Dierikx C, van Essen-Zandbergen A, van Pelt W, Mevius D. Characterization of multidrug-resistant, qnrB2-positive and extended-spectrum-beta-lactamase-producing *Salmonella* Concord and *Salmonella* Senftenberg isolates. *J Antimicrob Chemother* 2010; 65: 872–875.
28. Rakotonirina HC, Garin B, Randrianirina F, Richard V, Talarmin A, Arlet G. Molecular characterization of multidrug-resistant

extended-spectrum β-lactamase-producing Enterobacteriaceae isolated in Antananarivo, Madagascar. *BMC Microbiol* 2013; 13: 85.

29. Nilsen E, Haldorsen BC, Sundsfjord A *et al.*, The Norwegian Study Group on Invasive Enterobacter. Large IncHI2-plasmids encode extended-spectrum β-lactamases (ESBLs) in *Enterobacter* spp. bloodstream isolates, and support ESBL-transfer to *Escherichia coli*. *Clin Microbiol Infect* 2013; doi: 10.1111/1469-0691.12274.
30. Breurec S, Guessennd N, Timinouni M *et al. Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. *Clin Microbiol Infect* 2013; 19: 349–355.